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Supporting Information

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When the Leader Gets Loose: In Vivo Biosynthesis of a Leaderless Prenisin Is Stimulated by a *trans*-Acting Leader Peptide

Rustem Khusainov* and Oscar P. Kuipers^[a]

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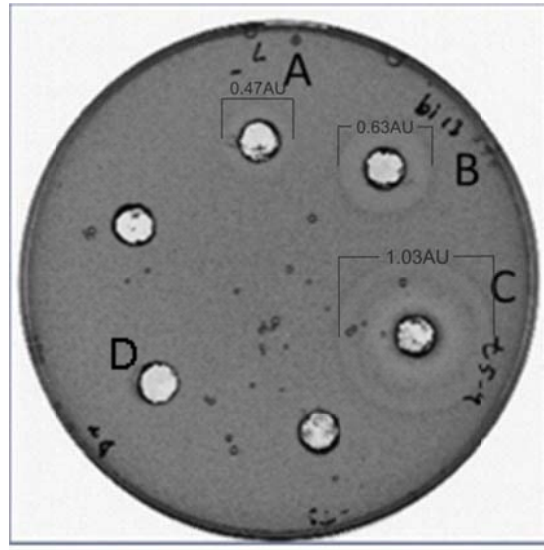


Figure S1. Antimicrobial activity assay of the Ni-NTA purified nisin variants. Nisin variants modified by NisBTC enzymes *in vivo* were purified and applied to the agar plate containing nisin-sensitive indicator strain *L. lactis* NZ9000 expressing NisPT. A) nisin variant NisA(24-57)-H6 matured without the leader was applied. B) The purified sample from the bicistronic system that expressed the leader and the propeptide part *in trans*, were applied. C) wild-type prenisin, used as a positive control. D) elution buffer with NisP was applied (negative control). A halo indicates the presence of active nisin processed by NisP to remove the nisin leader. AU: arbitrary units. The squared values of inhibition zone radii are proportional to $\ln(\text{concentration})$.

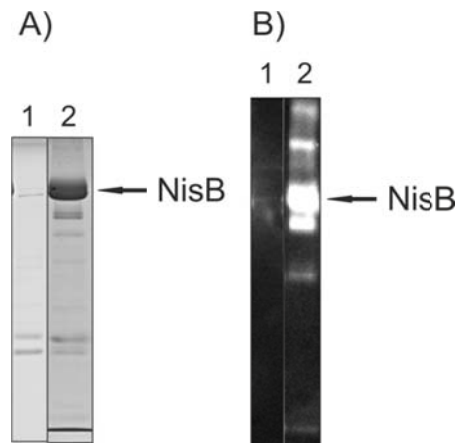


Figure S2. Elutions from Ni-NTA purification of His-tagged prenisin Lane 1: Strain containing wild type pIL3BTC vector and a leaderless NisA(24-57)-H6; lane 2: strain containing wild type pIL3BTC vector and NisA-H6; S2A. SDS-PAGE analysis of NisB co-purified with C-terminally extended His-tagged prenisin NisA-H6 S2B. Western blot of NisB co-purified with C-terminally extended His-tagged prenisin NisA-H6 detected by anti-NisB antibodies.

Primers used in this study:

pNZnisA-H6 vector,^[10] encoding for NisA-H6, was used as a template. The following set of primers was used to divide the *nisA* gene into two parts, for preparing the construct for the *in trans* expression of the nisin leader and the propeptide part.

R_nisA_leader_bicistr: 5'-TCAGC GTGGT GATGC ACCTG AATC-3'

F_nisA_leader_bicistr: 5'-ACATG ATCAA TTATA AGGAG GCACT CACCA TGATT
ACAAG TATTT CGCTA TGTAC ACCC-3'

In frame deletion of the region encoding for the nisin leader was made using the following set of primers.

F_del_leader_nisA: 5'-ATGAT TACAA GTATT TCGCT ATGTA CAC-3'

R_del_leader_nisA: 5'-GGTGA GTGCC TCCTT ATAAT-3'

Table S1. Dehydration of nisin variants containing a C-terminal extension GSIEGR with a His-tag, modified *in vivo* by NisBTC in *L. lactis*, isolated by Ni-NTA purification and analysed by MALDI-TOF mass spectrometry.

Mass [$M + H$] ⁺ w/o Met1 [Da]			
Mutant	Dehydration extent	Observed [Da]	Calculated [Da]
NisA(24-57)-H6	0 + Met	5046.34	5048
	1 + Met	5028.46	5030
	2 + Met	5010.51	5012
	3 + Met	4993.32	4994
	4 + Met	4975.31	4976
	5 + Met	4957.41	4958
	4 – Met	4844.51	4845
	3 – Met	4862.48	4863
NisA(1-23) and NisA (24-57)-H6 (<i>in trans</i>)	5 – Met	4826.42	4827
wild-type NisAH6	9 – Met	7109.79	7110
	8 – Met	7091.67	7092